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We have chosen the MISIIR promoter as the most appropriate ovarian promoter to direct expression of the cre protein in the ovarian surface epithelium. Transgenic MISIIR-cre mice have been received and a colony established at our facility.

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## INTRODUCTION

Ovarian cancer is the fifth most common cancer in women in the United States with a survival rate at late stage diagnosis of only 30-40%. More than 80% of these malignant tumors are of epithelial origin and yet very little is known about the ovarian surface epithelium. Efforts to understand the etiology and progression of ovarian cancer have been hampered by the lack of a good animal model. Recently, two laboratories have made significant headway in the establishment of a mouse model using very different approaches. The first successful ovarian cancer mouse model was reported in February 2002 by Orsulic *et al.* Because an ovarian surface epithelium (OSE)-specific promoter had yet to be identified, the authors isolated ovarian cells from transgenic mice expressing the avian retroviral receptor, TVA and transfected them *in vitro* with RCAS retroviruses encoding specific oncogenes. These ovarian cells were injected at subcutaneous, intraperitoneal, or ovarian sites where tumors resembling human ovarian carcinomas then developed. In March 2003 a more conventional approach for targeting the surface epithelium was reported by Connolly *et al.* The authors used the *MISIIR* promoter to drive expression of the transforming region (the T antigen) of SV40 in the OSE of transgenic mice to create ovarian tumors resembling human ovarian cancer that disseminate, form ascites, and express epithelial cell markers. Both models hold great promise for advancing the study of ovarian cancer pathogenesis. We will be building on the knowledge recently available, in particular the usefulness of the *MISIIR* promoter, to develop transgenic mice in which potentially oncogenic genes will be knocked out or knocked down.

## BODY

The third and final year of this proposal originally was November 1, 2002 to October 31, 2003; however, we requested and received a no cost extension for the final year until October 31, 2004 as we shifted the focus of the research. So although our third year will now officially start November 1, 2003, we have (1) made a significant change in our personnel and (2) made a final selection of the promoter to utilize to drive the cre protein in the surface epithelium. During this interim period we have hired Dr. Colleen Hough who started her appointment at the University of Utah late July 2003. Dr. Hough, who developed SAGE libraries from both normal OSE and ovarian tumors of epithelial origin at the NIH during her postdoctoral fellowship, has been initiating this change of direction.

### Task 1 *To identify a promoter that drives gene expression specifically in the mouse ovarian surface epithelium*

Our first task was to identify an appropriate promoter to drive transgenic genes in the OSE. We have exhaustively searched the literature as well as communicated with key investigators in the area of ovarian cancer and OSE promoters to understand the studies that have been completed and the resulting data so that we do not duplicate previous efforts. An example of such an effort has been reported by Tanyi *et al.* In an attempt to identify a promoter that shows high expression in ovarian surface epithelium cells or that is selectively expressed, they investigated four specific promoters after a review of the literature. Three potential epithelial cell-selective (hESE1, SLP1, OSP1) and one potential tumor-selective (hTERT) promoter were positioned upstream of a luciferase

reporter to determine the relative activity of these four promoters. The hTERT promoter exhibited the highest tumor selectivity but none of the promoters exhibited strict ovarian epithelium selectivity. However, our objective is to define a promoter that is expressed in normal OSE, so hTERT does not appear to be useful. Similarly, the usefulness of the  $\alpha$ -folate promoter was investigated but found to be associated in OSE only with tumor progression (Tomassetti et al.). Casado et al. have also reviewed transcriptional targeting strategies for the ovarian surface epithelium; however, either the promoters are not yet defined or there is a lack of tissue specificity.

Although we originally thought our first task would be to experimentally screen for OSE specific promoters, this task has been completed by several labs with a long history of research in ovarian cancer and an exhaustive study of OSE promoters is currently underway at the NIH. Building on the scrutiny of others, we have identified the MISIR promoter as the best candidate for our studies. In particular, the *in vivo* results reported by Connolly et al. are quite convincing of the activity and specificity of a 1204 bp promoter region of the murine MISIR gene. They demonstrated that 50% of the transgenic mice developed tumors which invade the omentum and form ascites as do human ovarian carcinomas. The tumors were determined to be of epithelial origin by the detection of cytokeratin proteins and the absence of  $\alpha$ -inhibin, a granulosa cell protein. In addition, the expression of the MISRII promoter was established in human ovarian cancer cell lines and ascites from patients diagnosed with ovarian carcinoma, implying that MISIR is expressed in the same cells that give rise to ovarian cancer (Masiakos et al.).

**Task 2** *Use the mouse model described by Connolly et al. to express genes of interest in the mouse ovarian surface epithelium*

Our objective is to utilize the murine MISIR promoter to drive cre protein in the ovarian surface epithelium and knockdown or knockout a series of potentially oncogenic genes in a tissue specific manner. Initially, mouse lines with floxed oncogenes will be crossed with the MISIR-driven cre line. The mice will be examined for the presence of OSE tumors. Lines with floxed genes can be crossed to assess possible cooperative interactions leading to tumor formation in the absence of multiple targeted genes.

The cloning vector carrying the MISIR promoter has been obtained from Dr. Denise Connolly (Fox Chase Cancer Center, Philadelphia, PA). In addition, transgenic mice expressing cre from this promoter have been obtained from Dr. JoAnn Richards (Baylor College of Medicine, Houston, Texas). To maximize the results within the next year, we will initially utilize available floxed lines such as BRCA1, p53, and Rb1, all of which have a potential role in ovarian cancer. Other candidates will include ZO-2 which our studies in year 1 and 2 of this grant identified in the OSE as well as the oncogenes used by Orsulic et al. to create ovarian tumors (c-myc, k-ras, and akt).

Although it is beyond the scope of the final year of this grant, we are anticipating capitalizing on RNAi technology to knockdown genes in the OSE. We are obtaining a vector that expresses dsRNA from a pol II promoter such as MISRII and cleaves the dsRNA to siRNA within the nucleus (pDECAP, Shinegawa and Ishii, 2003) for knockdown analyses. A vector will be constructed for each targeted gene.

### **Key research accomplishments**

We have identified the MISIIR promoter as the best promoter to drive OSE specific deletion of targeted genes, obtained the MISIIR promoter construct, and established a colony of transgenic MISIIR cre mice.

### **Reportable outcomes**

No reportable outcomes at the time of this report.

### **Conclusions**

A formal request for a one year no cost extension and a change in the research focus of the third year of this grant has been submitted and approved by the U.S. Army Medical Research Acquisition Activity office. Therefore, although this is the third annual report, our third year of research is just beginning. During this last year we have hired Dr. Colleen Hough, and she has completed a thorough analysis of the data currently available on OSE promoters. We are now positioned to begin crossing MISIIR cre transgenic mice with mouse lines carrying floxed oncogenes.

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